Human Ehrlichioses in Brazil: First Suspect Cases

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Brazilian spotted fever (BSF) rickettsiosis is the most common and recognized of the human rickettsioses in Brazil. It is difficult to establish the diagnosis of human rickettsiosis infection by routine microbiologic methods, creating a false idea that *Rickettsia* and *Ehrlichia* infections are rare and without importance. New tick-borne diseases, like Human Granulocytic Anaplasmosis (HGA) and Human Monocytic Ehrlichiosis (HME), have been described in many countries. These diseases can present symptoms similar to rickettsioses of the spotted fever group, and they are transmitted by ixodid ticks. The first two suspected cases of human ehrlichiosis in Brazil were first considered to be cases of BSF. The differential diagnosis was made at the Minas Gerais Rickettsiosis Public Health Laboratory. The clinical and laboratory findings, with positive serology for the HME agent, indicated suspected cases of human ehrlichioses in Brazil.
Key Words: Rickettsioses, human ehrlichioses, Brazilian spotted fever, Brazil.

Rickettsiae and *Ehrlichiae* were considered in the past as a form of life between viruses and bacteria. In truth they are Gram-negative bacteria that live inside the cell and do not grow out of the cell in their vectors or mammalian hosts [1,2]. With such a highly evolved niche, it is difficult to establish the diagnosis of human rickettsiosis infection by routine microbiologic methods, creating a false idea that *Rickettsia* and *Ehrlichia* infections are rare and without importance [3].

Brazilian spotted fever (BSF) rickettsiosis is the most common and recognized human rickettsiosis in Brazil. Recently, some of these bacteria previously considered as animal parasites, were recognized as pathogenic for humans, causing diseases such as human granulocytic anaplasmosis (HGA) and human monocytic ehrlichiosis (HME). These diseases can present symptoms similar to spotted fever rickettsiosis, and they are transmitted by ixodid ticks [4,5].

The American medical literature describes patients with human ehrlichiosis who usually visit a physician's office during the first week of the disease subsequent to an incubation period of 5 to 10 days after the tick bite [6]. The symptoms include fever, headache and myalgia. Other signs and symptoms include nausea, vomiting, diarrhea, cough and mental confusion. In contrast to BSF, the presence of a rash is less common in HME patients. However, renal failure, intravascular coagulation disorders, meningoencephalitis, adult respiratory distress syndrome, and coma may occur in severe cases. Pleocytosis in cerebrospinal fluid is associated with human ehrlichioses [3,7-9]. These diseases have not been described before in humans in

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Brazil, and *Ehrlichia* has not been described in arthropod vectors from Brazil. Evidence of antibodies reactive to *Ehrlichia chaffeensis* was reported for the first time in 2002 in a serologic survey of dogs from a spotted fever endemic region in Minas Gerais state [10]. The implementation of specific techniques that permit distinguishing between the different rickettsioses has been fundamental to elucidate infections such as ehrlichioses and other rickettsial diseases.

Materials and Methods

The suspected cases were detected by an epidemiological surveillance system in Minas Gerais state as suspected cases of BSF, and the sera of these patients were examined by indirect immunofluorescence assay [11]: Ten microliters of diluted sera were added to each well of the antigen slides for *R. rickettsii* and *E. chaffeensis*, which were incubated for 30 min in a humid chamber. The antigen used was obtained from the PANBIO Laboratory. The slides were rinsed once and washed in PBS (pH 7.1) for 10 minutes, twice. Fluorescein isothiocyanate-conjugated goat anti-human IgA, IgG and IgM (H+L) (Biolab Laboratories) was used at the optimal working dilution of 1/100 as the secondary antibody. After incubation for 30 min., the slides were rinsed once in PBS for 10 minutes, then washed in PBS (pH 7.1) containing Evans blue for 5 minutes, and then in sterile water for 5 more minutes. The slides were mounted with 90% glycerin in PBS under coverslips and observed under epifluorescence with an ultraviolet microscope (Zeiss, M C 80 DX) at 40X magnification. End point titers were determined by examination of serial two-fold dilutions of the reactive sera.

Results and Discussion

Case 1

A 39 year old man from Monte Carmelo Municipality, Minas Gerais state, Brazil was suspected to have BSF. The outset of the symptoms occurred on May 8, 2001, with fever, headache, nausea, vomiting, myalgia, conjunctivitis, respiratory insufficiency and renal failure. The patient had a clinical history of immunosuppression, pneumonia and chronic renal failure. He had been exposed to a dog that hunted rats.

Laboratory results on May 28, 2001: 1,600/mm^3white blood cells; 2,800,000/mm^3red blood cells; 8.8 g/dL hemoglobin; 25.8% hematocrit; and 42,000/mm^3 platelets. The IFA serologic reactions for BSF and murine typhus, macroagglutination test for leptospirosis, and ELISA for yellow fever and dengue revealed no antibodies. The presence of HME and HGA antibodies was determined by IFA, with the following results: IgM 1/64, IgG 1/128 and anti-human IgA, IgG and IgM (H+L) 1/512 to *Ehrlichia chaffeensis*. HGA antibodies were not detected.

Antibiotic therapy with chloramphenicol was initiated on May 29, with a good clinical response observed at 48 hours. A second sample of serum was not collected.

Case 2

A 20-year-old man lived in Ponte Nova Municipality, Minas Gerais State, Brazil. On May 15, 2001, he noted the onset of fever, headache, myalgia, conjunctivitis, nausea and vomiting. He developed icterus, respiratory insufficiency, renal failure, diarrhea, hepatomegaly, malnutrition and mental confusion by May 20. Laboratory results on May 20, 2001, revealed pleocytosis of the CSF, 240 IU/L oxalacetic transaminase, 53 IU/L pyruvic transaminase, 13.85 mg/dL direct bilirubin, and 22.61 mg/dL indirect bilirubin. The serologic reactions for BSF and murine typhus by IFA, leptospirosis by macroagglutination test, and yellow fever and dengue by ELISA revealed no antibodies; but antibodies against *E. chaffeensis* (IgM 1/64, IgG 1/256 and anti-human IgA, IgG and IgM (H+L) 1/64) were detected, with no reaction to HGA. The patient died on May 25, 2001.

These diagnoses of HME were made based on clinical and serologic results. The authors agree that hepatic enzyme changes in patient 2 are due to acute

Figure 1.

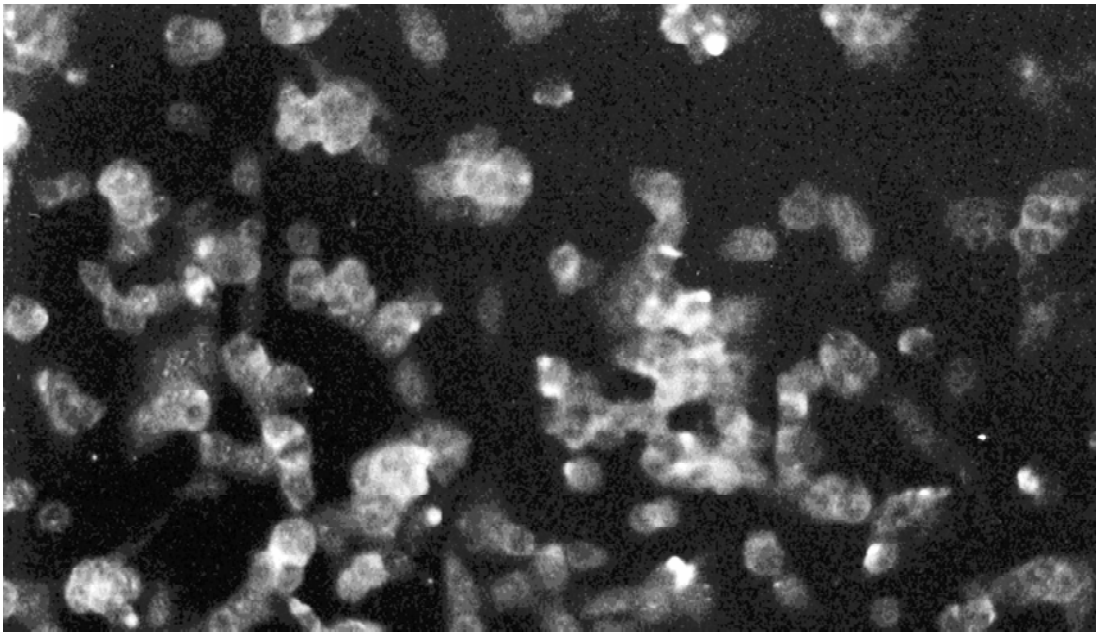
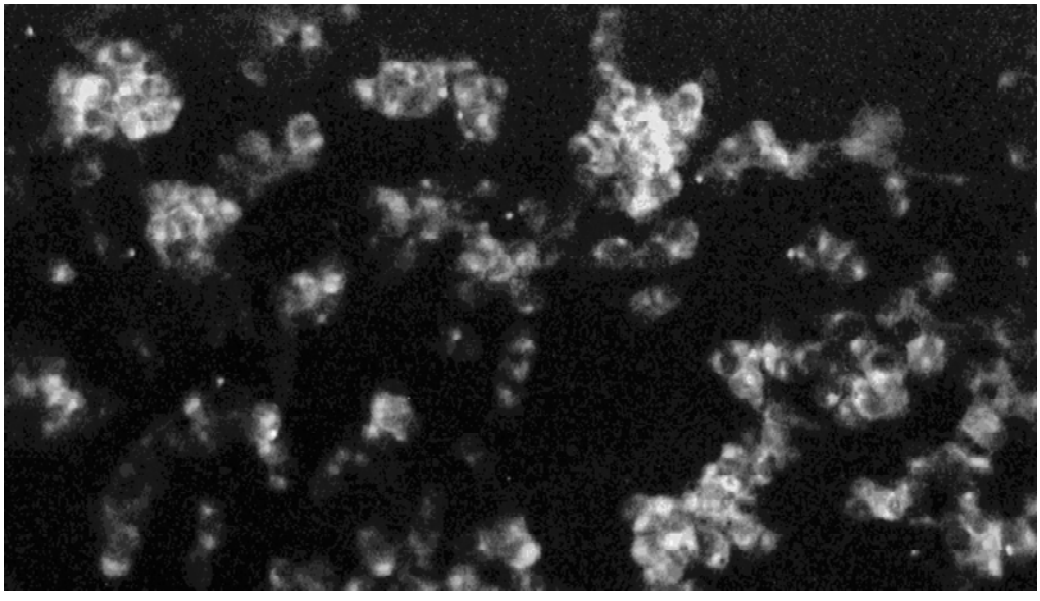


Figure 2.



illness. This patient had no history of previous hepatic or hematological diseases, and the possibility of hepatic renal syndrome being responsible for hepatic enzyme changes was also discarded based on clinical findings. The peripheral smears for morula were not reexamined because the physicians were not thinking of this diagnosis when the cases occurred. The serology does not confirm a diagnosis, but it can be suggestive. In our cases, the strong fluorescence by IFA to *E. chaffeensis*, the negative results for other rickettsioses and the significant titers of antibodies obtained for IgM and IgG to *E. chaffeensis* collaborate to place these cases in the category of suspected cases.

These first reported suspected cases of human ehrlichioses in Minas Gerais State, Brazil, show us the necessity of further studying the epidemiology of vectors and agents involved in this pathology, as well as in other rickettsioses. The finding of these agents in the environment increases the effectiveness of the diagnosis. Also, the correct diagnosis is important to initiate early treatment to significantly reduce the case fatality-ratio. Moreover, this could be important in differential diagnoses of hemorrhagic fevers.

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